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PERSPECTIVE

The Dark Side of the Immunohistochemical Moon: Industry

Alexander E. Kalyuzhny

Department of Immunocytochemistry and Elispot Assays, R&D Systems, Inc., Minneapolis, Minnesota

SUMMARY Modern biological research is dependent on tools developed and provided by commercial suppliers, and antibodies for immunohistochemistry are among the most frequently used of these tools. Not all commercial antibodies perform as expected, however; this problem leads researchers to waste time and money when using antibodies that perform inadequately. Different commercial suppliers offer antibodies of varying degrees of quality and, in some cases, are unable to provide expert technical support for the immunohistochemical use of their antibodies. This article briefly describes the production of commercial antibodies from the manufacturer's perspective and presents some guidelines for choosing appropriate commercial antibodies for immunohistochemistry. Additionally, the article suggests steps to establish mutually beneficial relationships between commercial antibody suppliers and researchers who use them. **(J Histochem Cytochem 57:1099–1101, 2009)**

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No matter how well-thought-out and solid the theory is, critical keys to success are research tools, and antibodies for immunohistochemistry (IHC) are among the most frequently used tools in modern biomedical research. A search of PubMed for articles that use immunohistochemistry and related terms reveals a 3-fold increase in articles that used immunostaining methods over the last 20 years (Figure 1). It is axiomatic, therefore, that the better the antibodies used for IHC, the higher the value and credibility of the generated scientific data.

For people who do a considerable amount of IHC, the world could be quite simple with just two sides to it: Us—industry, which makes the antibodies, and Them—the investigators of various caliber, expertise, and skill who are buying and using antibodies for their research. Unlike "Them," the "Us" side remains invisible for the most part, and our names are not usually known to Them. As for Us, we respect and need Them, but the Them have a more colorful spectrum of contradictory feelings toward Us: love, hate, relief, anger, and, in some cases, appreciation.

Correspondence to: Alexander E. Kalyuzhny, PhD, Department of Immunocytochemistry and Elispot Assays, RD Systems, Inc., 614 McKinley Place N.E., Minneapolis, MN 55413. e-mail: alex.kalyuzhny@rndsystems.com

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Making antibodies today is not as technically difficult as it was a decade ago, given the tools and reagents currently available to anyone. And this poses the question: why do researchers prefer using commercial antibodies rather than making their own? Is it not great to be your own "antibody boss" (instead of bowing to all those greedy companies who are making tons of money?), and even sell (that's what Bob from the corner lab on the seventh floor said the other day) simple, lyophilized buffer without any traces of IgG? There is no need to use ultrasensitive spy equipment to record such sentiments; just go to scientific meetings and walk by a poster line. Unlike complaints, antibody compliments resemble species on the verge of extinction: they are very rare, and super-quiet. The first answer to "why" is time: instead of waiting for months to make their own antibodies, investigators prefer spending their time generating data to use in publications and to meet grant application deadlines. The second answer is that making a good antibody is not so easy, and often akin to winning a lottery. Although we can control the manufacture of immunogens, their conjugation to select carrier proteins, and the boosting of host animals according to a perfectly crafted protocol, we are helpless in controlling the immune system of the host animal: antigen-presenting and B-cells of the host animal are actually running the show (for reference, see Parham 2000). This is why we may care more about the rabbits producing the best antibodies than we do about our best friends and family members.

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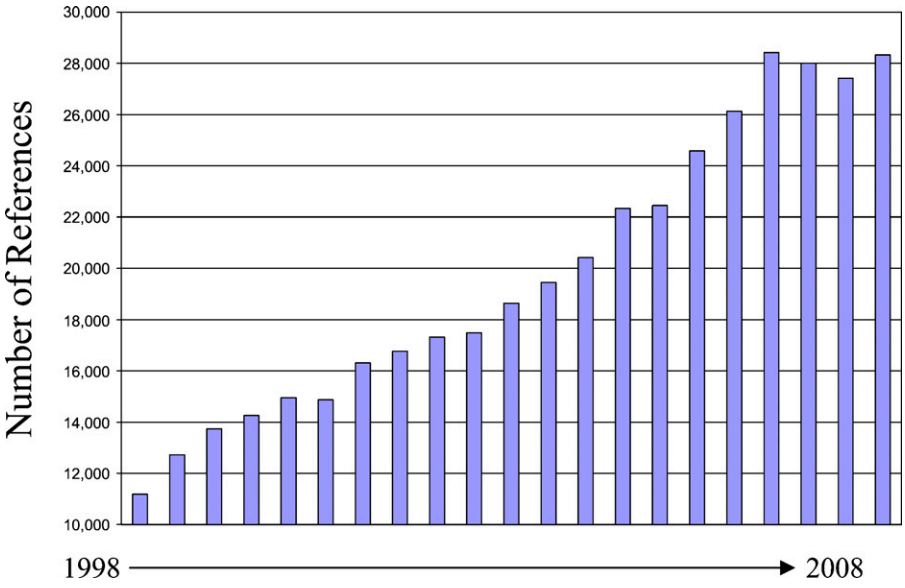

Figure 1 Increase in IHC publications over a 20-year period from 1998 through 2008. The number of IHC-related references was searched in PubMed by using the following string of keywords and operators: "immunocytochemistry" or "immunohistochemistry" or "immunofluorescence" and setting the limits for each year from January 1 to December 31.

Are there magic tricks to raising good antibodies for IHC? Yes, there are tricks, but they are far from being magic. In essence, they represent well-calculated and resource-consuming protocols that utilize numerous "know-how" solutions. Producing good commercial antibodies involves immunizing a large number of animals of different host species. Often it involves quite complicated workflow protocols, with quality control applied not only to manufactured antibodies but also to reagents and instruments used to make antibodies. Although the author of this perspective is not entitled to speak for the entire biotechnology industry, in the view of this author, it appears that receipt of a complaint from a customer is a very unpleasant scenario that hurts not only the self-esteem of the scientist who developed the antibody, but also the reputation of the company. And we all know that it is better to lose money than reputation. That is why companies invest (and if they don't, they should) a considerable amount of resources into validating antibodies before releasing them as products.

But what constitutes a good antibody? A good antibody is a specific one (any undergrad student knows this). And what is a specific antibody? A specific antibody is one that only recognizes the protein of interest. Antibodies are considered "junk" if they detect either wrong histological profiles and/or irrelevant bands on Western blots; this is a dead end, with nothing to argue about. Actually, I hate the word "junk" being used to refer to an antibody, because I know how much effort, time, and money go into making an antibody. And what if the antibody detects a specific protein plus traces of nonspecific ones? If I see labeling in motor neurons in the spinal cord as expected, should I worry about nonspecific labeling of a few astrocytes in the cerebral cortex? Going one step further, what should the ratio of specific to nonspecific signal be to conclude either that the antibody is good or that it should be dismissed as "junk"? Given different levels of histology and immunohistochemical experience, investigators often employ their own antibody acceptance criteria. And sometimes it appears that (rephrasing the popular old saying) "one researcher's junk can be another researcher's treasure". Unlike an IHC diagnostic (Trautman 1997; Hsi 2001; Whitmore 2004), there are no FDA-like guidelines for antibodies intended for research use only, giving a large degree of freedom in data interpretation and acceptance. For many IHC researchers, the only credible authority remains a commercial supplier of antibodies, and that is why investigators feel so frustrated, and even betrayed, when commercial antibodies do not work (Couchman 2009). The situation can turn particularly nasty if customers realize that there is no qualified technical or customer support. We can all agree that there should be a requirement for commercial suppliers to provide technical support as part of the assurance that their antibodies are suitable for the detection of a specified protein target.

On the other hand, it appears that scientists may not do enough research about commercial suppliers of antibodies. Which commercial source of antibodies should researchers choose? What questions should researchers ask when deciding on which door they should knock? In general, there are two types of antibody sellers: manufacturers and distributors. Manufacturers are (or should be) in full control of their production, covering every stage of antibody generation, including antigen design, antigen synthesis, immunization, antibody purification, testing, and quality control. Manufacturers are likely to have their own dis-

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tribution channels, selling directly, and also may supply some of their antibodies to distributors who do not have the capacity to make their own antibodies, simply serving as "value-added" resellers. Is it safe to buy from a distributor? Yes, it is, if such a distributor has a good working relationship with a manufacturer who helps with the troubleshooting for the distributor's customers. Customers should ask the distributor about his relationship with the antibody producer, to be on the safe side. Before ordering any antibodies for IHC, it would be a good idea to contact the company by phone or e-mail, and make sure it can provide qualified histological and immunohistochemical support. Having a good antibody for IHC is not only about getting a strong staining signal with low background, but also about knowing that the staining makes sense in terms of its histological and physiological relevance. Ordering an antibody is often not an event but a process. If you are not satisfied with your IHC staining, in many cases the company can help by offering a different lot or an antibody raised in a different species, or by suggesting better detection reagents that may or may not tweak the IHC protocol. In some cases, there is even a potential for customers to send their tissue sections to the antibody supplier for testing. The bottom line is this: do your preordering research and choose your commercial supplier wisely.

Lately, a few independent third-party test sites have emerged, offering their antibody validation services. Third-party antibody validation may sound like an attractive idea, but there is another side to the offer: what if the antibody tested and recommended by a third-party site does not work in customers' hands? Third-party test sites that have no access to antibody production and quality control records may not be able to solve the problem. Additionally, what if the third-party site mistakenly rejects an antibody that was well-characterized and approved by a commercial supplier? Who should the customer regard as an authority? It is obvious that such outcomes are confusing to both customers and commercial suppliers and can present serious problems.

How to solve the supplier–customer dilemma? One suggestion would be a requirement for antibody guidelines that require commercial suppliers to adhere to certain rules and customers to use antibodies according to provisions established within these guidelines with regard to staining conditions and controls. It would not be easy to set such guidelines, and would undoubtedly require considerable time and effort on the part of commercial suppliers and the National Institutes of Health (NIH). But there are guidelines on the use of lab animals; why not establish guidelines for the use of antibodies? This would require that commercial suppliers meet certain criteria, and could even include a ranking system for commercial suppliers that would help customers in their search for a reliable supplier. It might be expected that such guidelines could be created by a committee that includes both industry and basic science IHC experts, editors of peer-reviewed scientific journals with strong IHC emphasis, such as the *Journal of Histochemistry and Cytochemistry*, *The Journal of Comparative Neurology*, *The Journal of Neuroscience*, and *American Journal of Pathology*, to mention a few, and also NIH officials. This would make the actions of commercial suppliers more transparent, and help to establish trustworthy relationships with end-users of antibodies.

It is hard to imagine that progress in biological science would be possible without the tools developed by commercial suppliers. On the other hand, the success and prosperity of commercial suppliers would hardly be possible without the successful identification of novel molecules by researchers in academic labs. Therefore, the successful use of antibodies by end-users is in the best interests of both commercial antibody suppliers and researchers. Their success is our success: existing tools help in the discovery of new molecules, which leads to the need for new research tools that commercial suppliers can develop.

It should not be "Us" and "Them," because we are all in the same boat, heading for the same destination.

The views and opinions expressed in this perspective are the author's own and not those of R&D Systems, Inc.

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